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AWARD NUMBER: W81XWH-05-1-0592

TITLE: PSMA-Targeted Nano-Conjugates as Dual-Modality (MRI/PET) Imaging Probes for the Non-Invasive Detection of Prostate Cancer

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REPORT DATE: January 2010

TYPE OF REPORT: Final Addendum

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
1 January 2010	Final Addendum	15 Sep 2009 – 14 Dec 2009

4. TITLE AND SUBTITLE

PSMA-Targeted Nano-Conjugates as Dual-Modality (MRI/PET) Imaging Probes for the Non-Invasive Detection of Prostate Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-05-1-0592

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Texas Southwestern Medical Center at Dallas
Dallas, TX 75390

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The goal of this project is to develop dual modality imaging probes for the detection of prostate cancer by doping radioisotopes to iron oxide nanoparticles, so that the sensitivity and specificity of prostate cancer diagnosis could be significantly improved. During the funding period, a facile approach was developed to prepare gamma- or positron emitting nuclides incorporated SPIO nanoparticles (NUSPIONs) for dual modality imaging of prostate cancer. In this three-month extension, we successfully developed the logistics of importing 74As/GeO2 targets from Europe and developed a standardized operation protocol for the isotope separation and 74AsI3 preparation. Using 74AsI3, we have applied our developed methodology to synthesize 74As incorporated detxtran-coated iron oxide nanoparticles (~ 20 nm) and performed PET-CT and MRI evaluation in a PC-3 tumor mouse model. The PET/MRI dual modality imaging results clearly demonstrated the practicality of the methodology conceived and developed in this project for non-invasive detection of prostate cancer. Further research is warranted to move this project forward.

15. SUBJECT TERMS
None provided.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	8	19b. TELEPHONE NUMBER *(include area code)*

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Introduction

As mentioned in my letter of request for this three-month extension, we ordered two shipments of As-74 from Belgium for this project. In the Revised SOW and Timeline, we specified one task to be performed in this three-month period:

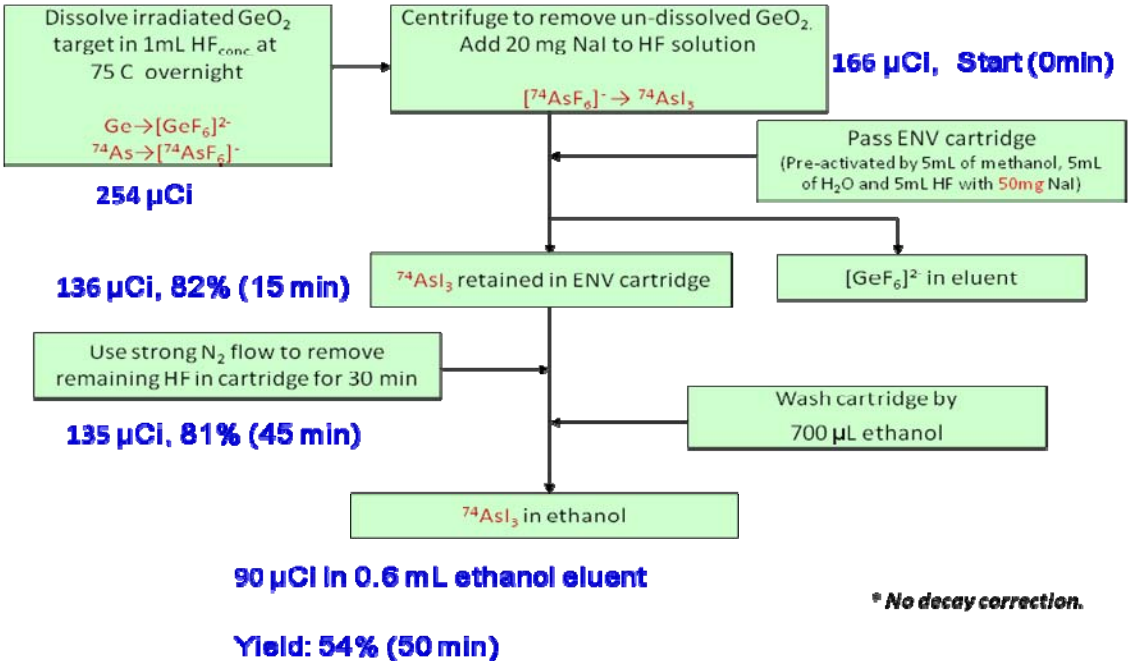
Months 49 – 51 (Task 2/Subtask 2):

We have requested two shipments of ^{74}As from Belgium to "Accomplish the small animal MRI and PET imaging evaluation of the two nano-conjugates based on ^{74}As-incoporated iron oxide nanoplatform."

Research Progress in the three-month extension:

1. Target Processing of Irradiated ^{74}As/GeO_2 powder for $^{74}AsI_3$ Preparation

Two shipments of ^{74}As/GeO2 were received in the three-month extension from the VUB-Eenheid Cyclotron Facility of Vrije Universiteit Brussels, Belgium. The activity of ^{74}As was 3.93 mCi for the first shipment (11/30/09) and 4.3 mCi for the second shipment (12/7/09). The ^{74}As activity was in 350 mg of irradiated GeO_2 powder. After several trials, we have established a standardized operation procedure for the separation of ^{74}As from GeO_2 and preparation of $^{74}AsI_3$ in reasonable radioactivity recovery rates as shown in **Scheme 1**.



Scheme 1. Schematic ^{74}As/GeO_2 target processing and preparation of $^{74}AsI_3$

2. Incorporation of ^{77}As into dextran coated iron oxide nanoparticles

The synthetic procedure was adapted from the standard protocol of incorporating ^{77}As into iron oxide nanoparticles (see the 1st annual report) as follows.

The Fe_3O_4 nanoparticles incorporated with $^{74}As^{3+}$ were obtained via co-precipitation of Fe^{2+}, Fe^{3+}, and radioisotope in a solution phase system by the addition of a base solution. First, 35.7mg of $FeCl_3 \cdot 6H_2O$, 13.1mg of $FeCl_2 \cdot 4H_2O$, and 2.0g of Dextran T10 were dissolved in 30 mL deoxygenated Milli-Q water by intensive stirring under a protection of N_2 gas flow until a homogenous transparent light yellow solution with a pH value of 3 was obtained. The following reactions were carried out in a three-neck flask under the protection of a N_2 gas with flow rate at 2 LPM. To 20 ml of the Fe^{3+}/Fe^{2+}/dextran solution, 70.4 μCi of $^{74}AsI_3$ in 1.0 mL ethanol was added. The flask was immersed in an ice/water box and kept stirring at 800 rpm for 15 min. Then 1 mL of 6.55 wt.% ammonium hydroxide solution was added slowly and continuously at a rate of 6mL/h by the control of a syringe pump. The light yellow solution slowly turned into dark green then to black. After stirring the solution for 30 min, the flask was transferred from the ice/water box to an oil bath and subsequently heated up to 78°C in an hour. A pH value of 7 was measured after the solution cooled down to room temperature. The cross linking of dextran shell was carried out by adding 6 mL of 5 M NaOH and 3mL of epichlorohydrin. The reaction was allowed to proceed for 2 h at room temperature. The resulted reaction mixture with total radioactivity of 68.4 μCi was concentrated by centricon filter tubes to remove the non-reacted ions and excess dextran molecules. The nanoparticles (DLS diameter: 20 nm) were washed several times with water until less than 1 μCi of radioactivity was detected from the filtrate. The yield of ^{74}As incorporation was 30.5% after decay correction. The injection doses were prepared in 400 μL of solution with 11.7 μCi of ^{74}As-activity and 0.1 mmol/kg iron.

3. PET-CT and MRI Imaging of Prostate Cancer with ^{74}As-incorporated Iron Oxide Nanoparticles

Tissue Culture and Animal Model: All animal studies were performed in compliance with guidelines set by the UT Southwestern Institutional Animal Care and Use Committee. The PC-3 cell line was obtained from the American Type Culture Collection (ATCC, Manassas, VA). PC-3 cells were cultured in T-media (Invitrogen, Carlsbad, CA) at 37 °C in an atmosphere of 5% CO_2 and were passaged at 75 % confluence in P150 plates. T-media was supplemented with 5% Fetal Bovine Serum (FBS) and 1 × Penicillin/Streptomycin. PC-3 cells were harvested from monolayer using PBS and trypsin/EDTA, and suspended in T-media with 5% FBS. The cell suspension was then mixed 1:1 with Matrigel™ and injected subcutaneously (2.5×10^6 cells per injection, injection volume 100 μL) into both front flanks of

SCID mice. After injection, animals were monitored three times a week by general observations. The tumor was noticed to grow in the first week and allowed to grow three weeks to reach a palpable size for the imaging experiments.

MicroPET-CT: Small animal PET-CT imaging studies were performed on a Siemens Inveon PET-CT Multimodality System (Siemens Medical Solutions Inc., Knoxville, TN, USA) when the prostate cancer xenografts reached the similar size of approximate 100 mm^3. The injected dose was 11.7 μCi of ^{74}As-activity in 400 μL PBS (three injections); and the iron dose was kept at 0.1 mmol/kg for MRI evaluation.

Ten minutes prior to imaging, the animal was anesthetized using 3% Isofluorane at room temperature until stable vitals were established. Once the animal was sedated, the animal was placed onto the imaging bed under 2% Isofluorane anesthesia for the duration of the imaging. The microCT imaging was acquired at 80 kV and 500 A with a focal spot of 58 μm. The total rotation of the gantry was 360^o with 360 rotation steps obtained at an exposure time of approximately 235 ms/frame. The images were attained using a CCD readout of 4096 × 3098 with a bin factor of 4 and an average frame of 1. Under low magnification the effective pixel size was 103.03 μm. Total microCT scan time was approximately 6 min. CT images were reconstructed with a down sample factor of 2 using Cobra Reconstruction Software. The PET imaging was acquired directly following the acquisition of CT data. The PET tracers were injected intravenously via the tail vein. Static PET scans were performed at 15 h post injection for 45 min. PET images were reconstructed using Fourier Rebinning and Ordered Subsets Expectation Maximization 3D (OSEM3D) algorithm. Reconstructed CT and PET images were fused and analyzed using the Siemens Inveon Research Workplace (IRW) software.

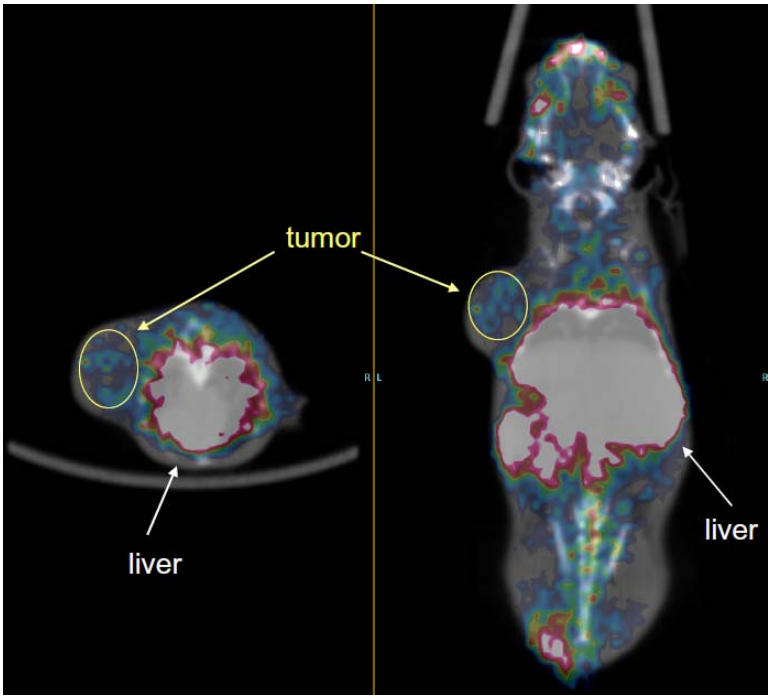


Figure 1. PET-CT images of a PC-tumor bearing mouse injected with ^{74}As-incorporated iron oxide nanoarticles. Left: transaxial; right: coronal. The imaging was acquired for 45-min after 15 h post-injection.

The PET-CT images of a mouse injected with ^{74}As-incorporated iron oxide nanoparticles are shown in Figure 1. The PC-3 tumor showed

reasonable uptake of the nanoparticles at 12-h post-injection. Due to the low injection of ^{74}As-activity, the imaging was acquired for 45-min. The tumor PET contrast (signal/noise) may be improved by increasing the injection dose of ^{74}As-activity. The high liver accumulation reflects the nature of nanoparticles in vivo, indicating the necessity of nanoparticle surface modifications.

Mouse MRI. The MRI evaluation was performed on a 4.7 T Varian MRI system equipped with a homemade coil after the completion of PET-CT imaging. The mice were anesthetized by inhalation of 1.5-2.0 % isoflorane in air. Multi-slice T_2 maps were obtained using a spin echo sequence.

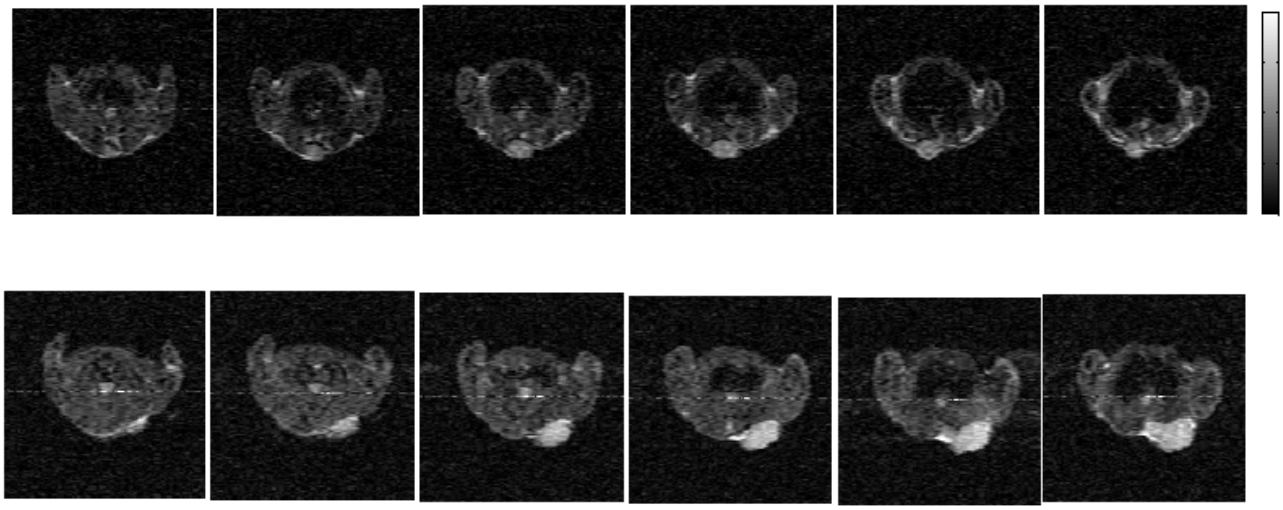


Figure 2. Multi-slice T_2 MR images of a PC-3 tumor-bearing mouse injected with ^{74}As-incorporated iron oxide nanoarticles at the iron dose of 0.1 mmol/kg (top panel) and a PC-3 tumor-bearing mouse without the nanoparticle injection (low panel).

As shown in Figure 2, we can clearly see the T_2 shortening effect resulted from the tumor (top) with iron oxide nanoparticle accumulation as compared to the control tumor (bottom). Therefore we performed ROI analysis on a slice-by-slice basis using homebuilt Matlab routines and the T_2 values from all voxels of each tumor were pooled for statistical comparison using either students' t-test or 1-way ANOVA by GraphPad Prism® program. We observed statistically significant decrease (compared to Control tumor) in the mean T_2 value from 52.7 ±14.0 ms to 43.6 ±12.5 ms in mice injected with iron oxide nanoparticles.

Key Research Accomplishments

In addition to the accomplishments summarized in my annual and final reports, we have achieved the following ones in this three-month extension:

1) Successfully separated ^{74}As from $^{74}As/GeO_2$ targets and established a standard procedure to synthesize $^{74}AsI_3$.
2) Successfully applied our developed methodology to incorporate ^{74}As into the core of detxtran-coated iron oxide nanoparticles (~ 20 nm).
3) Successfully performed PET-CT and MRI evaluation of the ^{74}As-incorporated iron oxide nanoparticles in a PC-3 tumor mouse model. PET imaging of PC-3 tumor with ^{74}As-incorporated incorporated iron oxide nanoparticles showed a marginal success, while T_2 weighted MRI results were much more significant.

Reportable Outcomes

One manuscript will be prepared based on the results obtained in this extended period.

Conclusions

In this three-month extension, we have accomplished the preparation of ^{74}As-incorporated iron oxide nanoparticles and successfully used the PET/MRI dual modality imaging probes to visualize prostate cancer in a subcutaneous tumor mouse model. While this part of work was impeded by the availability of ^{74}As, to date we have shown the practicality of using ^{74}As-incorporated iron oxide nanoparticles for PET/MRI dual modality imaging of prostate cancer. Therefore I believe we have satisfactorily accomplished the goal set for this project.